

AMENDMENT TO THE CLAIMS

This listing of claims replaces all previous listing of claims pending in this application.

Listing of Claims

1. (Currently amended) A conjugate comprising a β -arrestin protein and a ~~label, detectable molecule~~.
2. (Currently amended) The conjugate of claim 1, wherein ~~said label the detectable molecule~~ is an optically detectable molecule.
3. (Currently amended) The conjugate of claim 1, wherein ~~said label the detectable molecule~~ is Green Fluorescent Protein.
4. (Original) A nucleic acid construct comprising an expression cassette, which construct comprises, in the 5' to 3' direction, a promoter and a nucleic acid segment operatively associated therewith, the nucleic acid segment comprising a sequence encoding a β -arrestin protein and a sequence encoding a detectable molecule.
5. (Original) A nucleic acid construct according to claim 4, wherein the nucleic acid sequence encoding a detectable molecule encodes an optically detectable molecule.
6. (Original) A nucleic acid construct according to claim 4, wherein the nucleic acid sequence encoding a detectable molecule encodes Green Fluorescent Protein.
7. (Original) A nucleic acid construct according to claim 4, said construct further comprising a plasmid.
8. (Original) A host cell containing a nucleic acid construct according to claim 4.
9. (Original) A nucleic acid construct according to claim 4 which is a DNA construct.
10. (Original) A nucleic acid construct according to claim 4 which is an RNA construct.
11. (Original) A DNA construct according to claim 4 wherein said promoter is a β -arrestin promoter.
12. (Original) A host cell comprising a nucleic acid molecule, the nucleic acid molecule comprising in the 5' to 3' direction, a promoter operable in the host cell, a nucleic acid sequence encoding a β -arrestin protein and a nucleic acid sequence encoding a detectable molecule, the encoding nucleic acid sequences operably linked to the promoter.

13. (Original) A cell according to claim 12, wherein the cell is a mammalian cell.
14. (Original) A cell according to claim 12, wherein the cell is selected from HEK-293 cells and COS cells.
15. (Original) A cell according to claim 12, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.
16. (Original) A substrate having deposited thereon a host cell according to claim 12.
17. (Withdrawn – currently amended) A method of assessing G protein coupled receptor (GPCR) pathway activity under test conditions, comprising: a) providing a test cell that expresses a GPCR, and contains a conjugate ~~according to claim 1; comprising a β -arrestin protein and a visually detectable molecule~~; b) exposing the test cell to a known GPCR agonist under test conditions; and then c) detecting translocation of ~~said conjugate the detectable molecule~~ from the cytosol of the test cell to the membrane edge of the test cell; wherein the translocation of the detectable molecule in the test cell indicates activation of the GPCR pathway.
18. (Withdrawn) A method according to claim 17 wherein the test condition is the presence in the test cell of a test kinase.
19. (Withdrawn) A method according to claim 17 wherein the test condition is the presence in the test cell of a test G-protein.
20. (Withdrawn) A method according to claim 17 wherein the test condition is exposure of the test cell to a test ligand.
21. (Withdrawn) A method according to claim 17 wherein the test condition is coexpression in the test cell of a second receptor.
22. (Withdrawn – currently amended) A method for screening a β -arrestin protein or fragment thereof for the ability to bind to a phosphorylated GPCR, comprising: a) providing a cell that: i) expresses a GPCR; and ii) contains a conjugate ~~according to claim 1; comprising a test β -arrestin protein and a visually detectable molecule~~; b) exposing the cell to a known GPCR agonist; and then c) detecting translocation of ~~said conjugate the detectable molecule~~ from the cytosol of the cell to the membrane edge of the cell; wherein translocation of ~~said conjugate the detectable molecule~~ in the test cell indicates β -arrestin protein binding to the phosphorylated GPCR.

23. (Withdrawn – currently amended) A method for screening a test compound for G protein coupled receptor (GPCR) agonist activity, comprising: a) providing a cell expressing a GPCR and containing a conjugate according to claim 1; comprising a β -arrestin protein and a visually detectable molecule; b) exposing the cell to the test compound; and then c) detecting translocation of said conjugate ~~the detectable molecule~~ from the cytosol of the cell to the membrane edge of the cell; wherein movement of said conjugate ~~the detectable molecule~~ from the cytosol to the membrane edge of the cell after exposure of the cell to the test compound indicates GPCR agonist activity of the test compound.

24. (Withdrawn) A method according to claim 23 wherein the cell expresses a known GPCR.

25. (Withdrawn) A method according to claim 23 wherein the cell expresses an unknown GPCR.

26. (Withdrawn) A method according to claim 23 wherein the cell expresses an odorant GPCR.

27. (Withdrawn) A method according to claim 23 wherein the cell expresses a β -adrenergic GPCR.

28. (Withdrawn – currently amended) A method according to claim 23 wherein said conjugate ~~the detectable molecule~~ is optically detectable.

29. (Withdrawn – currently amended) A method according to claim 23 wherein said conjugate comprises ~~the detectable molecule is~~ Green Fluorescent Protein.

30. (Withdrawn) A method according to claim 23 wherein the cell is a mammalian cell.

31. (Withdrawn) A method according to claim 23, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

32. (Withdrawn) A method according to claim 23, wherein the cell normally expresses a GPCR.

33. (Withdrawn) A method according to claim 23, wherein the cell has been transformed to express a GPCR not normally expressed by such a cell.

34. (Withdrawn) A method according to claim 23, where the test compound is in aqueous solution.

35. (Withdrawn) A method according to claim 23, where the cells are deposited on a substrate.

36. (Withdrawn – currently amended) A method of screening a sample solution for the presence of an agonist to a G protein coupled receptor (GPCR); comprising: a) providing a cell expressing a GPCR and containing a conjugate according to claim 1; ~~the conjugate comprising a β -arrestin protein and a visually~~

~~detectable molecule;~~ b) exposing the cell to a sample solution; and then c) detecting translocation of said conjugate ~~the detectable molecule~~ from the cytosol of the cell to the membrane edge of the cell; wherein movement of said conjugate ~~the detectable molecule~~ from the cytosol to the membrane edge of the cell after exposure of the cell to the sample solution indicates the sample solution contains an agonist for a GPCR expressed in the cell.

37. (Withdrawn) A method according to claim 36 wherein the cell expresses a known GPCR.

38. (Withdrawn) A method according to claim 36 wherein the cell expresses an unknown GPCR.

39. (Withdrawn) A method according to claim 36 wherein the cell expresses an odorant GPCR.

40. (Withdrawn) A method according to claim 36 wherein the cell expresses a β -adrenergic GPCR.

41. (Withdrawn – currently amended) A method according to claim 36 wherein said conjugate ~~the detectable molecule~~ is optically detectable.

42. (Withdrawn – currently amended) A method according to claim 36 wherein said conjugate comprises ~~the detectable molecule~~ is Green Fluorescent Protein.

43. (Withdrawn) A method according to claim 36 wherein the cell is a mammalian cell.

44. (Withdrawn) A method according to claim 36, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

45. (Withdrawn – currently amended) A method of screening a test compound for G protein coupled receptor (GPCR) antagonist activity, comprising: a) providing a cell expressing a GPCR, and containing a conjugate according to claim 1; ~~the conjugate comprising a β -arrestin protein and a visually detectable molecule;~~ b) exposing the cell to a test compound; c) exposing the cell to a GPCR agonist; and d) detecting translocation of said conjugate ~~the detectable molecule~~ from the cytosol of the cell to the membrane edge of the cell; where exposure to the agonist occurs at the same time as, or subsequent to, exposure to the test compound, and wherein movement of said conjugate ~~the detectable molecule~~ from the cytosol to the membrane edge of the cell after exposure of the cell to the test compound indicates that the test compound is not a GPCR antagonist.

46. (Withdrawn) A method according to claim 45 wherein the cell expresses a known GPCR.

47. (Withdrawn) A method according to claim 45 wherein the cell expresses an odorant GPCR.

48. (Withdrawn) A method according to claim 45 wherein the cell expresses a β -adrenergic GPCR.

49. (Withdrawn – currently amended) A method according to claim 45 wherein said conjugate the detectable molecule is optically detectable.

50. (Withdrawn – currently amended) A method according to claim 45 wherein said conjugate comprises the detectable molecule is Green Fluorescent Protein.

51. (Withdrawn) A method according to claim 45 wherein the cell is a mammalian cell.

52. (Withdrawn) A method according to claim 45, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

53. (Withdrawn) A method according to claim 45, where the cells are deposited on a substrate.

54. (Withdrawn – currently amended) A method of screening a test compound for G protein coupled receptor (GPCR) antagonist activity; comprising: a) providing a cell expressing a GPCR and containing a conjugate according to claim 1; ~~the conjugate comprising a β -arrestin protein and a visually detectable molecule~~; b) exposing the cell to a GPCR agonist so that translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell occurs; c) exposing the cell to a test compound; where exposure to the agonist occurs prior to exposure to the test compound, and wherein movement of said conjugate the detectable molecule from the membrane edge of the cell to the cytosol after exposure of the cell to the test compound indicates that the test compound has a GPCR antagonist activity.

55. (Withdrawn – currently amended) A method according to claim 54 wherein said conjugate the detectable molecule is optically detectable.

56. (Withdrawn – currently amended) A method according to claim 54 wherein said conjugate comprises the detectable molecule is Green Fluorescent Protein.

57. (Withdrawn) A method according to claim 54 wherein the cell is a mammalian cell.

58. (Withdrawn) A method according to claim 54, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

59. (Withdrawn) A method according to claim 54, where the test compound is in aqueous solution.

60. (Withdrawn) A method according to claim 54, where the cells are deposited on a substrate.

61. (Withdrawn – currently amended) A method of screening a cell for the presence of a G protein coupled receptor (GPCR); comprising: a) providing a test cell, said test cell containing a conjugate according to claim 1; comprising a β -arrestin protein and a visually detectable molecule; b) exposing the test cell to a solution containing a GPCR agonist; and c) detecting translocation of said conjugate the detectable molecule from the cytosol of the cell to the membrane edge of the cell; wherein movement of said conjugate the detectable molecule from the cytosol to the membrane edge of the test cell after exposure of the test cell to the GPCR agonist indicates that the test cell contains a GPCR.

62. (Withdrawn – currently amended) A method according to claim 61 wherein said conjugate the detectable molecule is optically detectable.

63. (Withdrawn – currently amended) A method according to claim 61 wherein said conjugate the detectable molecule is Green Fluorescent Protein.

64. (Withdrawn) A method of screening a plurality of cells for those cells which contain a G protein coupled receptor (GPCR); comprising: a) providing a plurality of test cells, said test cells containing a conjugate according to claim 1; comprising a β -arrestin protein and a visually detectable molecule; b) exposing the test cells to a known GPCR agonist; and c) detecting those cells in which said conjugate the detectable molecule is translocated from the cytosol of the cell to the membrane edge of the cell; wherein movement of said conjugate the detectable molecule from the cytosol to the membrane edge of a cell after exposure to the GPCR agonist indicates that the cell contains a GPCR for that known GPCR agonist.

65. (Withdrawn – currently amended) A method according to claim 64 wherein said conjugate the detectable molecule is optically detectable.

66. (Withdrawn – currently amended) A method according to claim 64 wherein said conjugate comprises the detectable molecule is Green Fluorescent Protein.

67. (Withdrawn) A method according to claim 64 wherein the plurality of test cells are contained in a tissue.

68. (Withdrawn) A method according to claim 64 wherein, the plurality of test cells are contained in an organ.

69. (Withdrawn) A method according to claim 64 wherein step (b) comprises exposing the test cells to a plurality of known GPCR agonists.

70. (Withdrawn) A substrate having deposited thereon a plurality of cells, said cells expressing a GPCR and containing a conjugate, the conjugate comprising a β -arrestin protein and a detectable molecule.

71. (Withdrawn) A substrate according to claim 70, wherein the detectable molecule is an optically detectable molecule.

72. (Withdrawn) A substrate according to claim 70, wherein the detectable molecule is Green Fluorescent Protein.

73. (Withdrawn) A substrate according to claim 70 wherein the cell is a mammalian cell.

74. (Withdrawn) A substrate according to claim 70, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

75. (Withdrawn) A substrate according to claim 70, wherein the cell expresses an olfactory GPCR.

76. (Withdrawn) A substrate according to claim 70, wherein the cell expresses a β -adrenergic GPCR.

77. (Withdrawn) A substrate according to claim 70, wherein the substrate is made of a material selected from glass, plastic, ceramic, semiconductor, silica, fiber optic, diamond, biocompatible monomer, and biocompatible polymer materials.

78. (Original) An apparatus for determining GPCR activity in a test cell, comprising: means for measuring indicia of the intracellular distribution of a detectable molecule; and a computer program product comprising a computer readable storage medium having computer-readable program code means embodied in said medium, said computer-readable program code means comprising: computer-readable program code means for determining whether the indicia of the distribution of the detectable molecule in the test cell indicate concentration of the detectable molecule at the cell membrane, based on comparison to the measured indicia of the intracellular distribution of said detectable molecule in said control cell.

79. (Original) An apparatus for determining GPCR activity in a test cell, comprising: means for measuring indicia of the intracellular distribution of a detectable molecule in at least one test cell at multiple time points, and a computer program product comprising a computer readable storage medium having computer-readable program code means embodied in said medium, said computer-readable program code means comprising: computer-readable program code means for determining whether the indicia of the distribution of the detectable molecule in the test cell at multiple time points indicates translocation of the detectable molecule to the cell membrane.

80. (Original) An apparatus for determining GPCR activity in a test cell, comprising: means for measuring indicia of the intracellular distribution of a detectable molecule in at least one test cell; and a computer program product comprising a computer readable storage medium having computer-readable program code means embodied in said medium, said computer-readable program code means comprising: computer-readable program code means for determining whether the indicia of the distribution of the detectable molecule in the test cell indicates concentration or the detectable molecule at the cell membrane, based on comparison to pre-established criteria.

81. (Original) The apparatus of claim 78, where said indicia of the intracellular distribution of a detectable molecule are optical indicia.

82. (Original) The apparatus of claim 78, wherein said measuring means comprises means for measuring fluorescent intensity.

83. (Original) The apparatus of claim 78, wherein the molecule to be detected is a fluorescently detectable molecule, and wherein said step of measuring indicia of the intracellular distribution of said detectable molecule comprises measuring fluorescence signals from the test and control cells.

84. (New) The conjugate according to claim 1, wherein said label is a fluorescent label.

85. (New) The conjugate according to claim 1, wherein said label is a colorimetric label.

86. (New) The conjugate according to claim 1, wherein said label is a radioactive label.

87. (New) The conjugate according to claim 1, wherein said label is an electron-dense label.